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Genetic parameters and genotype by environment interaction for cold tolerance, body weight and survival of the Pacific white shrimp *Penaeus vannamei* at different temperatures

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ABSTRACT

The inability of *Penaeus vannamei* to tolerate low temperatures is of major economic concern in temperate climates, as it reduces their growing season and leads to over-winter mortality. In this study, the heritability of body weight and survival under two grow-out temperature conditions, genotype by environment interaction for body weight and survival at different grow-out temperatures, and the heritability of cold tolerance traits of *P. vannamei* were investigated and estimated. The heritability estimates for body weight in normal controls (CK) and low-temperature groups (LTT) were, respectively, moderate (0.3191 ± 0.0622) and low (0.1368 ± 0.0889), and those for survival were all low (0.1094 ± 0.0265 and 0.0598 ± 0.0229, respectively). The genetic correlations between the CK and LTT groups for body weight and survival were 0.4788 ± 0.2073 and 0.2968 ± 0.2284 respectively, and both of them showed significant differences from unity ($P < 0.05$). Furthermore, their *K*-values were all higher than 0.50, which indicated that the genotype-by-environment interaction effect was substantial in both growth and survival at different temperatures. The cold tolerance heritability estimated from a cold temperature challenge (18 °C to 8 °C) using cooling degree hours for each individual (CDH) and survival rate of each family at half lethal time (SR_{50}) were low (0.0258 ± 0.0205 and 0.0211 ± 0.0196 respectively), and negative genetic correlations between these cold tolerance traits and body weight (−0.7702 ± 0.4583 and −0.8253 ± 0.4553 respectively) were also estimated from the cold challenge. However, they showed no statistically significant differences from zero. Thus, more research needs to be conducted on the more accurate heritability of cold tolerance trait and genetic correlations between traits in *P. vannamei* to further improve the achievement of breeding goals.

1. Introduction

The Pacific white shrimp, *Penaeus vannamei*, whose natural distribution is along the Pacific coast of the western American continent from Mexico to Peru, has become the primary cultivated species in various locations in the eastern hemisphere (Funge-Smith and Briggs, 2005; Wang and Chen, 2005). Between and within different countries, shrimp are cultured in a large variety of environmental and culture conditions. *P. vannamei* grow between 25 °C and 35 °C (Ponce-Palafox et al., 1997), and they stop feeding at temperatures lower than 18 °C. The temperature of seawater in temperate and some subtropical regions, such as northern China, where *P. vannamei* is widely cultured, can fall much lower than this limit (18 °C), which may be significant because temperature is one of the most important natural factors affecting the defense mechanisms of crustaceans. For example, shrimp farming in China has been adversely affected by winter mortality for several decades, especially in 2008 (Qiu et al., 2011). Thus, a genetic improvement program concentrated on cold tolerance in *P. vannamei* is necessary and important, as it can increase production, prolong the grow-out period and decrease the cost of shrimp culture industry in temperate and some subtropical regions.

Several selective breeding programs are being developed for *P. vannamei*, and both ANOVA and restricted maximum likelihood (REML) methods have been used to estimate heritability for the shrimp's growth-related, survival and disease-resistance traits (Goyard et al., 1999; Argue et al., 2002; Pérez-Rostro and Ibarra, 2003a, b; Gitterle et al., 2005a, b; Gjedrem, 2005; Castillo-Juárez et al., 2007; Cock et al., 2009; Luan et al., 2012; Luan et al., in press). It has been shown that environmental changes can affect the response to selection in breeding programs. Phenotypic plasticity in the growth response to different densities is accompanied by a significant genotype by environment interaction, evidenced by a change in heritability between environments and by a genetic correlation less than one for a unique

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trait between environments (Ibarra and Famula, 2008). Although there are few reports on genetic parameters of cold tolerance traits in *P. vannamei*, significant genetic effects on cold tolerance have already been found for both intra- and interspecific crosses in a number of aquatic animals, such as the Mozambique tilapia *Oreochromis mossambicus*, blue tilapia *O. aureus*, and their F_1 hybrids (Cnaani et al., 2000); Nile tilapia *O. niloticus* (Charo-Karisa et al., 2005); red drum *Sciaenops ocellatus* (Ma et al., 2007) and *Hucho taimen* (Wang et al., 2011). Such findings highlight the potential for genetic improvement of cold tolerance in cultured aquatic animals. Assessing the potential for genetic improvement of cold tolerance in *P. vannamei* requires knowledge of the genetic parameters of this trait. There has not, however, been any previous report on the genetic parameters and genotype by environment interaction for body weight, survival and cold tolerance traits at different water temperatures in *P. vannamei* until now.

We present here an estimate of heritability for body weight and survival after growing 55 families under two grow-out temperature conditions, an analysis of the genotype by environment interaction for body weight and survival between different grow-out temperatures and an estimate of heritability for the cold tolerance of *P. vannamei*. Our goal was to understand whether different water temperatures affect estimates of those genetic parameters for body weight and survival in Pacific white shrimp (*P. vannamei*) and to elucidate the heritability of their cold tolerance traits.

2. Materials and methods

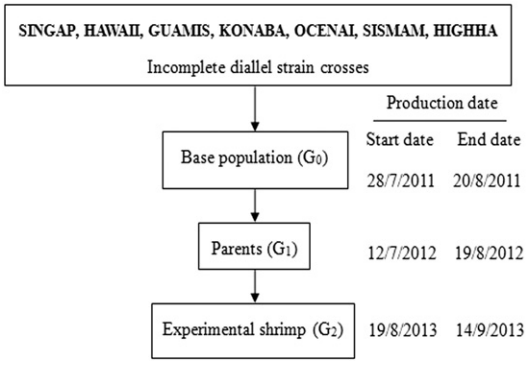
2.1. Historical background and shrimp production

All experimental procedures were conducted at the Mariculture Research Station of Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, located in the suburbs of Qingdao (latitude 36°20′32.22″N, longitude 120°39′1.93″E, altitude 3.04 m), Shandong province, China. The base population (G_0) was derived from seven commercial *P. vannamei* strains (founder population) using an incomplete diallel cross experiment in 2011. The seven strains were separately introduced from Shrimp Improvement System Pte. Ltd (SINGAP), Shrimp Improvement System Hawaii LLC (HAWAII), University of Guam, CNAS (GUAMIS), Kona Bay Marine Resources, Waimea Aquatic Lab (KONABA), The Oceanic Institute (OCENAI), Shrimp Improvement System Florida (SISMAM) and High Health Aquaculture Inc (HIGHHA) in June 2011. Their origins were Singap, Oahu, Guam, Kauai, Oahu, Miami and Hawaii, respectively. The pedigrees of individuals in the seven strains were unknown. During subsequent generations (G_1 to G_2), the selection population was closed, and generations were discrete. G_1 to G_2 generations were produced using a full-sib/half-sib mating design. 69 sires and 66 dams from the base population (G_0) were used in 2012 to produce parental shrimps (G_1), which contained 72 full-sib families. A total of 55 full-sib families were produced as experimental shrimp (G_2) from 52 sires and 43 dams of parental shrimps (G_1). These consisted of 28 half-sib families. There existed an age difference of 24, 39 and 27 days for G_0, G_1 and G_2 generation respectively, which was caused by the different mating and spawning time. The pedigree of all of the individuals was known and was used to construct a relationship matrix. The number of sires, dams, full-sib families, half-sib families and the shrimp production scheme is shown in Fig. 1.

For G_0 to G_2 generation, each breeding candidate was reared separately in a small net cage (0.25 m × 0.25 m × 0.25 m) labeled with a four-digit identity code. Breeding candidates could then be individually identified using the cage ID. Female and male parents were carefully chosen to maximize mating success. Females with orange colored ovaries that occupied a large area of the cephalothorax were preferred. Males with a healthy appearance and filled spermatophore were obtained for mating with the sexually receptive females. Each full-sib group of fertilized eggs was hatched in a separate tank. The eggs hatched after approximately one day, and a random sample of approximately 3000 nauplii from each full-sib family were stocked in separate larvae-culture tanks. Hatched nauplius larvae passed through six nauplius stages, three zoea stages, three mysis stages and metamorphosis to post-larva in approximately ten days. A random sample of approximately 200 post-larvae from each family was transferred into larger tanks (3 m^3) for separate rearing until the last family reached tagging size (post-larval ages of 73–95 days). Then, the shrimps from each family were tagged using a visible implant elastomer, with each family received a unique tag code.

2.2. Growth and survival tests under different water temperatures

The tagged experimental shrimps (G_2) were allowed to recover from tag stress for seven days. Then, from each full-sib family, 48 randomly chosen tagged individuals were distributed equally to four 9 m^2 concrete ponds as normal controls (CK), and another 40 samples were similarly allocated among four other 7.5 m^2 concrete ponds and served as the low-temperature experimental group (LTT). The mean body weight of each family was recorded at the same time. The water temperature was monitored each hour from the beginning to the end of the experiment. For the CK group, the water temperature was kept at 28 °C ± 1 °C during the experiment, whereas the temperature of LTT group was reduced at a rate of 2 °C/day to 18 °C and kept at 18 °C ± 1 °C to the end of the experiment. Apart from the hourly temperature measurements, dissolved oxygen (DO), pH, total ammonia, nitrate and nitrite were measured once a day, using a WTWR multi 340i meter and HACH kits. Dissolved oxygen ranged between 6.1 and 10.3 mg/l, the pH ranged from 7.9 to 8.3, ammonia levels ranged from



	Production date		Sires	Dams	Full-sib families	Half-sib families	
	Start date	End date				Paternal	Maternal
Base population (G_0)	28/7/2011	20/8/2011	114	108	130	32	37
Parents (G_1)	12/7/2012	19/8/2012	69	66	72	6	11
Experimental shrimp (G_2)	19/8/2013	14/9/2013	52	43	55	6	22

Fig. 1. Schematic presentation of production of experimental shrimps from seven commercial *P. vannamei* strains from Singapore and the United States. Abbreviations: SINGAP-strain form Shrimp Improvement System Pte. Ltd; HAWAII—strain form Shrimp Improvement System Hawaii LLC; GUAMIS—strain form University of Guam, CNAS; KONABA—strain form Kona Bay Marine Resources, Waimea Aquatic Lab; OCENAI—strain form OCENAI The Oceanic Institute; SISMAM—strain form Shrimp Improvement System Florida; HIGHHA—strain form High Health Aquaculture Inc.